Pharmacokinetics of a long-acting ceftiofur crystalline-free acid formulation in Asian elephants (*Elephas maximus*)

Michael J. Adkesson, DVM; Randall E. Junge, MS, DVM; Matthew C. Allender, DVM, MS; Tomás Martín-Jiménez, DVM, PhD

**Objective**—To determine the pharmacokinetics of a long-acting formulation of ceftiofur, ceftiofur crystalline-free acid (CCFA), following SC injection to Asian elephants (*Elephas maximus*).

**Animals**—11 adult Asian elephants.

**Procedures**—Each elephant received CCFA (6.6 mg/kg, SC) in the area caudoventral to the base of an ear. Blood samples were collected from an ear vein immediately prior to and at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours after CCFA administration. Plasma concentrations of desfuroylceftiofur acetamide (the acetamide derivative of ceftiofur) were measured via ultrahigh-pressure liquid chromatography–tandem mass spectrometry. Data were analyzed via a noncompartmental pharmacokinetics approach.

**Results**—The mean ± SD maximum plasma concentration of desfuroylceftiofur acetamide was 1.36 ± 0.74 µg/mL and was detected at 47.18 ± 31.30 hours. The mean ± SD area under the curve from time 0 to infinity was 227.8 ± 55.8 µg•h/mL, and the mean residence time from time 0 to infinity was 158.2 ± 90.2 hours. The terminal elimination half-life associated with the slope of the terminal phase had a harmonic mean ± pseudo-SD of 83.36 ± 30.01 hours.

**Conclusions and Clinical Relevance**—Elephants tolerated CCFA at a dose of 6.6 mg/kg, SC, well. Dosing recommendations will depend on the mean inhibitory concentration of ceftiofur for each bacterial pathogen. Desfuroylceftiofur acetamide concentrations remained >0.25 µg/mL for the entire 168-hour study period, which suggested CCFA would provide clinically relevant antimicrobial activity against certain pathogens for 7 to 10 days. *(Am J Vet Res 2012;73:1512–1518)*

Antimicrobials are routinely administered to elephants for the treatment of gastroenteritis, pneumonia, skin abscesses, foot abscesses, and various other primary and secondary bacterial infections. Elephant-specific pharmacokinetic data exist only for a small number of antimicrobials, of which few have a broad spectrum of antimicrobial activity.1–8 Results of previous pharmacokinetic research involving elephants indicate that extrapolations of drug doses solely on the basis of body mass are not always accurate and the metabolism of a drug in elephants may differ from the metabolism of the same drug in another species.9

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>Area under the plasma concentration–time curve</td>
</tr>
<tr>
<td>AUC∞</td>
<td>Area under the plasma concentration–time curve from time 0 to infinity</td>
</tr>
<tr>
<td>AUClast</td>
<td>Area under the plasma concentration–time curve from time 0 to the last measured concentration</td>
</tr>
<tr>
<td>CCFA</td>
<td>Ceftiofur crystalline-free acid</td>
</tr>
<tr>
<td>CFAE</td>
<td>Ceftiofur free-acid equivalent</td>
</tr>
<tr>
<td>Cmax</td>
<td>Peak plasma concentration</td>
</tr>
<tr>
<td>DCA</td>
<td>Desfuroylceftiofur acetamide</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>MIC</td>
<td>Mean inhibitory concentration</td>
</tr>
<tr>
<td>MRT</td>
<td>Mean residence time</td>
</tr>
<tr>
<td>Tmax</td>
<td>Time to peak plasma concentration</td>
</tr>
<tr>
<td>UPLC</td>
<td>Ultrahigh-pressure liquid chromatography</td>
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</table>

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Ceftiofur is a third-generation, parenterally administered cephalosporin with activity against many gram-positive, gram-negative, and anaerobic bacteria and is generally resistant to ß-lactamases. A crystalline-free acid formulation of ceftiofur suspended in a caprylic-capric triglyceride and cottonseed oil base has been developed for the treatment of respiratory infections in cattle caused by Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni (formerly Haemophilus somnis).10 Results of multiple studies involving cattle indicate that a single SC dose of CCFA is a safe and effective treatment for respiratory disease11–14 and infectious keratoconjunctivitis.15 In cattle, CCFA is administered as a single dose of 6.6 mg/kg, SC, in an ear, from where it is slowly absorbed and provides plasma concentrations that exceed the defined efficacy threshold for 5 to 8 days.17,18

Numerous challenges are associated with antimicrobial administration to elephants. Therefore, a long-acting antimicrobial with activity against many common bacterial pathogens that requires a minimal number of injections would be beneficial. The purpose of the study reported here was to evaluate the pharmacokinetics of CCFA following SC administration to captive adult Asian elephants (Elephas maximus).

Materials and Methods

Animals — The study reported here was approved by the Saint Louis Zoo Institutional Animal Care and Use Committee. Eleven (4 males and 7 females) adult Asian elephants from the Saint Louis and Oregon Zoos were included in the study. All elephants were healthy, in good body condition, and ranged in age from 11 to 43 years (mean ± SD, 28.1 ± 11.6 years; median, 27 years). Elephants were housed in their usual enclosures, and no alterations were made to diet or social groupings. Daily routines for the elephants were modified only as necessary to facilitate blood sample collection. Because of staff and safety constraints related to working with elephants, sample collection was conducted on only 1 to 2 elephants at any given time. A physical examination, CBC, and serum biochemical analysis was performed on each elephant prior to and at the conclusion of the study to determine health status. Elephants were weighed and visually examined daily for adverse reactions (eg, hypersensitivity reaction, injection site reaction, and abscess formation).

CCFA administration and sample collection — Each elephant received a single dose of CCFA* (6.6 mg/kg) SC under the loose skin immediately caudal to the base of an ear (caudal to the ramus of the mandible) with a 14- or 16-gauge needle attached to a 60-mL syringe via IV tubing. The volume of CCFA that was administered to each elephant was calculated on the basis of that elephant’s weight, which was measured immediately prior to injection. The mean ± SD body weight for the study elephants was 3,761 ± 1,375 kg (median, 3,407 kg). Therefore, the mean volume of CCFA injected was 124 mL (median, 107 mL), and the entire volume of CCFA was administered as 1 injection at a single site.

All elephants had been trained by means of behavioral conditioning to allow blood collection. Blood samples (4 to 6 mL) were collected into vials containing lithium heparin from an ear vein via a 20- or 23-gauge needle or butterfly catheter and syringe. Blood was collected immediately prior to (time 0) and at 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours after CCFA administration. Blood samples were refrigerated immediately after collection. Within 30 minutes after collection, blood samples were centrifuged for 10 minutes, and then plasma was harvested and frozen at −80°C until analysis.

Analytic method — Plasma concentrations of DCA (the acetamide derivative of ceftiofur) were measured in plasma via a validated UPLC–tandem mass spectrometry assay with an automated platform.19 Briefly, ceftiofur and desfuroylceftiofur-related metabolites were extracted from elephant plasma after a reduction step with the addition of 1,4-dithioerythritol solution. Stable labeled ceftiofur (ceftiofur-d3; molecular weight, +3) was used as the internal standard to control for derivatization and column extraction efficiency. Desfuroylceftiofur was captured on solid-phase extraction columns in a 96-well format after a 30-minute thioester reduction step at 50°C. The columns were washed with 0.1M ammonium acetate, and desfuroylceftiofur was derivatized with iodoacacetamide to form DCA. The DCA was then removed from the column via a 30:70 (vol/vol) mixture of acetonitrile and 0.01M ammonium acetate with 0.1% trifluoroacetic acid into a 96-well analytic plate. The eluent was diluted with an equal volume of 0.01M ammonium acetate with 0.1% trifluoroacetic acid, which provided a final injection equal to isotropic UPLC conditions (15% acetonitrile and 85% 0.01M ammonium acetate [0.1% trifluoroacetic acid]). Injections (10 µL) were made by means of a UPLC system with a flow rate of 0.5 mL/min and a total run time of 2.0 minutes. For detection of DCA by tandem mass spectrometry, the instrument was set to operate in positive ion mode with an electrospray ionization source. Positive ions were monitored in the multiple reaction monitoring mode with precursor product ion pairs of 487 to 241 for DCA. Standard regression of calibration standards was accomplished via quadratic regression of the ratio of analyte to the internal standard with 1/X² weighting (by the inverse of the squared observations). The lower LOQ for this assay was 10.0 ng/mL and the upper LOQ was 10.0 g/mL. Samples with DCA concentrations > 10 µg/mL were diluted in the appropriate matrix and reanalyzed. Samples were assayed in 3 analytic batches. For each batch, additional replicates of fortified quality control samples were assayed in duplicate. The interday coefficient of variability ranged between 0.8% and 3.3%, and the interday bias ranged between −10.0% and 8.8%. The intraday coefficient of variability ranged between 1.56% and 10.74%, and the intraday bias ranged between −10.6% and 9.4%.
Pharmacokinetic analyses—Plasma concentration data for DCA were analyzed for each elephant via a noncompartmental approach implemented with a pharmacokinetic software package. The \( C_{\text{max}} \) and \( T_{\text{max}} \) were recorded directly after examination of the data. The slope of the terminal phase was determined via log-linear regression including data from at least 4 sampling times. The terminal half-life was calculated as 0.693 divided by the slope of the terminal phase. The AUC \(_{0-\text{last}} \) was calculated via the log-linear trapezoidal rule. The AUC \(_{\text{last}} \) was calculated as the value of the last measured plasma DCA concentration divided by the slope of the terminal phase and then summed with the value of AUC \(_{0-\text{last}} \). The extrapolated fraction of the AUC \(_{0-\text{last}} \) was calculated as \((\text{AUC}_{0-\text{last}} - \text{AUC}_{\text{last}})/\text{AUC}_{0-\text{last}}\) \(\times 100\). The area under the first moment curve from time 0 to the last measured concentration of DCA and the area under the first moment curve from time 0 to infinity were calculated via the log-linear trapezoidal rule. The MRT from time 0 to the last measured concentration of DCA was calculated as the area under the first moment curve from time 0 to the last measured concentration of DCA divided by the AUC \(_{0-\text{last}} \). Similarly, the MRT from time 0 to infinity was calculated as area under the first moment curve from time 0 to infinity divided by the AUC \(_{\text{last}} \). The extravascular systemic clearance was calculated as the dose of CCFA/AUC \(_{\text{last}} \) and the extravascular apparent volume of distribution was calculated as extravascular clearance corrected for bioavailability divided by the slope of the terminal phase. Pharmacokinetic parameters were reported as the arithmetic mean ± SD, median, and range.

### Results

#### Animals

All animals remained healthy throughout the study. All results of CBCs and serum biochemical analyses for blood samples obtained prior to and at the conclusion of the observation period (168 hours after CCFA administration) were within established reference limits. Three elephants developed reactions at the CCFA injection site. A 25-year-old female elephant developed a raised, indurated plaque (13 X 18 X 2 cm) around the CCFA injection site that lasted for approximately 3 months and migrated ventrally without enlargement. Evaluation of fine-needle aspirates of the plaque revealed a sterile, mixed inflammatory response. Treatment of the plaque with warm water hydrotherapy had no apparent effect, but adverse systemic effects were not recorded and the plaque resolved completely. A 15-year-old female elephant developed a similar, but smaller indurated plaque (10 X 15 cm) around the CCFA injection site that also resolved without complication. A 36-year-old female elephant developed a 3-cm round swelling at the CCFA injection site approximately 1 month after the injection. This swelling was lanced, and approximately 12 mL of caseous material was removed from a well-encapsulated abscess. Aerobic culture of the caseous material yielded no growth. No adverse systemic effects were recorded, and the area healed without complication.

#### Pharmacokinetic parameters

Pharmacokinetic parameters were summarized (Table 1) and the mean plasma DCA concentration-time curve was plotted (Figure 1) following SC administration of CCFA to 11 captive Asian elephants (Elephas maximus).

### Table 1—Mean ± SD, median, range, and coefficient of variation for pharmacokinetic parameters obtained by noncompartmental analysis after SC administration of a single dose of CCFA (6.6 mg/kg) to 11 captive Asian elephants (Elephas maximus).

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (µg/mL)</td>
<td>1.36 ± 0.74</td>
<td>1.19</td>
<td>0.54–2.80</td>
<td>54.64</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (h)</td>
<td>47.18 ± 31.3</td>
<td>36.0</td>
<td>12.0–96.0</td>
<td>66.34</td>
</tr>
<tr>
<td>( \lambda_z ) (h(^{-1}))</td>
<td>0.008 ± 0.003</td>
<td>0.009</td>
<td>0.003–0.012</td>
<td>37.17</td>
</tr>
<tr>
<td>( t_{\text{av}} ) (h)</td>
<td>83.36 ± 30.01</td>
<td>80.34</td>
<td>58.00–257.53</td>
<td>36.00</td>
</tr>
<tr>
<td>AUC (_{\text{mod}} ) (µg h/mL)</td>
<td>145.77 ± 63.72</td>
<td>123.23</td>
<td>72.68–255.36</td>
<td>43.03</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{extrap}} ) (µg h/mL)</td>
<td>227.76 ± 55.75</td>
<td>226.85</td>
<td>150.16–322.26</td>
<td>24.48</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{last}} ) (µg h/mL)</td>
<td>4,573.82</td>
<td>3,677.94</td>
<td>1,951.86–11,185.67</td>
<td>65.45</td>
</tr>
<tr>
<td>( \lambda_z ) (h(^{-1}))</td>
<td>0.009 ± 0.003</td>
<td>0.007</td>
<td>0.003–0.012</td>
<td>37.17</td>
</tr>
<tr>
<td>AUC (_{\text{extrap}} ) (%)</td>
<td>29.55 ± 16.17</td>
<td>23.42</td>
<td>14.60–62.03</td>
<td>54.73</td>
</tr>
<tr>
<td>AUMC (_{\text{inf}} ) (µg h(^2)/mL)</td>
<td>10,855.7 ± 4,149.8</td>
<td>8,272.1</td>
<td>6,538.2–16,853.5</td>
<td>38.23</td>
</tr>
<tr>
<td>AUMC (_{\text{inf}} ) (µg h(^2)/mL)</td>
<td>35,647.1 ± 19,952.2</td>
<td>32,995.9</td>
<td>13,720.5–80,250.4</td>
<td>56.00</td>
</tr>
<tr>
<td>MRT (_{\text{av}} ) (h)</td>
<td>76.57 ± 8.79</td>
<td>74.98</td>
<td>63.63–89.71</td>
<td>11.48</td>
</tr>
<tr>
<td>MRT (_{\text{av}} ) (h)</td>
<td>159.24 ± 90.20</td>
<td>115.78</td>
<td>91.37–366.07</td>
<td>57.00</td>
</tr>
<tr>
<td>CV/F (mL/kg)</td>
<td>30.7 ± 8.14</td>
<td>29.09</td>
<td>20.48–43.95</td>
<td>26.52</td>
</tr>
<tr>
<td>V/F (mL/kg)</td>
<td>4,573.82 ± 2,993.72</td>
<td>3,677.94</td>
<td>1,951.86–11,185.67</td>
<td>65.45</td>
</tr>
</tbody>
</table>

*For 2 elephants, the plasma DCA concentration increased from the time of CCFA administration to 20 minutes after injection in a pattern similar to that of the other study elephants, but then the DCA concentration plateaued and did not decrease substantially for the remainder of the observation period. Therefore, only values for \( C_{\text{max}} \) and AUC \(_{\text{mod}} \) could be calculated for those 2 elephants. Thus, the values for \( \text{AUC}_{\text{extrap}} \) and AUC \(_{\text{extrap}} \) represent data obtained from all 11 study elephants, whereas the values for the remaining parameters represent data obtained from only 9 elephants. Harmonic mean = pseudo-SD.Coefficient of variation of pseudo-SD.

\( \text{AUC}_{\text{extrap}} \) = Extrapolated fraction of the AUC from time 0 to infinity. \( \text{AUMC}_{\text{inf}} \) = Area under the first moment curve from time 0 to the last measured concentration. \( \lambda_z \) = Slope of the terminal phase. MRT \(_{\text{av}} \) = Mean residence time from time 0 to the last measured concentration. MRT \(_{\text{av}} \) = Mean residence time from time 0 to infinity. \( t_{\text{av}} \) = Terminal elimination half-life associated with the slope of the terminal phase. V/F = Extravascular volume of distribution corrected for bioavailability.
Asian elephants. For 2 elephants, DCA concentrations increased from the time of CCFA administration to 20 hours after injection in a pattern similar to that for the other study elephants, but then the DCA concentration plateaued and did not decrease substantially for the remainder of the observation period. The AUC0–last results for those 2 elephants were 80.3 and 72.9 \( \mu g/\text{h/mL} \), which were the lowest AUC0–last results obtained among all the elephants and much lower than the mean AUC0–last (145.8 \( \mu g/\text{h/mL} \)) for the study population. Those 2 elephants also had the longest \( T_{\text{max}} \) (96 hours for both elephants) and lowest \( C_{\text{max}} \) (0.536 \( \mu g/mL \)) among the study population during the observation period. Because the plasma DCA concentration did not decrease significantly during the observation period for those 2 elephants, the remaining pharmacokinetic parameters for those 2 individual elephants could not be calculated. Therefore, data obtained for those 2 elephants were included only in the calculations of the mean \( T_{\text{max}} \), \( C_{\text{max}} \), and AUC0–last. The calculations of the means for the remaining pharmacokinetic parameters included data only from the remaining 9 elephants.

Absorption of CCFA following SC administration to Asian elephants was slow; the \( C_{\text{max}} \) mean \( \pm SD \), 1.36 \( \pm 0.74 \mu g/mL \) for DCA was not achieved until 47 hours (mean \( \pm SD \), 47.18 \( \pm 31.30 \) hours) after injection. Measurable concentrations of DCA in plasma were still detected at 168 hours after CCFA administration, the time at which blood sample collection was discontinued. The mean \( \pm SD \) AUC0–last was 227.8 \( \pm 55.8 \mu g/\text{h/mL} \) (median, 226.9 \( \mu g/\text{h/mL} \); range, 150.2 to 322.3 \( \mu g/\text{h/mL} \)). The mean \( \pm SD \) slope of the terminal phase was 0.008 \( \pm 0.003 \) h\(^{-1}\), and the terminal elimination half-life associated with the slope of the terminal phase (terminal half-life) had a harmonic mean \( \pm \text{pseudo-SD} \) of 83.36 \( \pm 30.01 \) hours. The mean \( \pm SD \) MRT from time 0 to infinity was 138.24 \( \pm 90.20 \) hours (median, 119.78 hours; range, 91.37 to 366.07 hours).

**Discussion**

All traditional routes of antimicrobial administration are possible in elephants, but the large size of elephants can make drug administration challenging. Oral drug administration can be difficult, particularly with unpalatable drugs, because of the large volume that is often required. Rectal administration of certain drugs can result in therapeutic plasma concentrations, but it is not an effective route of administration for all drugs. Furthermore, rectal administration of drugs is time-consuming and only possible in well-trained elephants. Elephants can be sensitive to injections despite their large size and thick skin, although many captive elephants have been trained to allow voluntary injections by both IV and IM routes. However, generally large volumes of injectable drugs are required, and repeated injections may not be tolerated even by well-trained elephants. Because IM injection of drug volumes > 15 to 25 mL/site is not recommended, the administration of a complete dose of a drug to an elephant often requires multiple injections. Thus, some form of restraint is often necessary for elephants when they are administered injectable drugs to ensure the safety of the animal management staff, veterinarians, and elephants. Abscesses at injection sites are not uncommon in elephants and generally cause minimal clinical problems. However, the risk of abscess formation increases when multiple injections are administered and abscesses can become problematic. Similarly, multiple IV injections increase the risk of formation of venous thrombi.

Results of a study conducted to evaluate the pharmacokinetics of ceftiofur sodium in elephants indicate that ceftiofur is a safe and effective antimicrobial for the treatment of susceptible bacterial infections but requires frequent IM or IV injections. We hypothesized that SC administration of a long-acting ceftiofur formulation such as CCFA to elephants would result in a slow release of the active form of ceftiofur (DCA) from the injection site and thereby decrease the number and frequency of injections required for treatment.

In veterinary medicine, drug doses are often extrapolated from results of research involving 1 species for application in another species; unfortunately, this approach has not worked well for elephants. Horses are generally considered to be the species most similar to elephants, but differences in body mass, metabolism, circulation time, density of capillaries per unit of a specific tissue, respiratory gas exchange surface, drug clearance, and other factors make extrapolations of drug doses from horses to elephants challenging. Results of pharmacokinetic studies indicate that doses of drugs established in other species are not appropriate for use in elephants. Furthermore, the absorption and elimination of long-acting medications may vary substantially by species, and dosages determined via species-specific data are recommended.

Ceftiofur is widely distributed in extracellular fluid but does not penetrate well into the CSF unless meningi.
Gangliosidosis is present.\textsuperscript{17} Ceftiolur is rapidly metabolized into its active metabolite, desfurolyceftiolur. Because desfurolyceftiolur is not stable in vivo, MIC data for ceftiolur is based solely on the parent drug, although ceftiolur, desfurolyceftiolur, and desfurolyceftiolur conjugates all exert antimicrobial activity in vivo.\textsuperscript{17,23}

The effectiveness of a single dose of CCFA is dependent on the MIC of ceftiolur for the targeted pathogen. Because CCFA is a time-dependent antimicrobial, the efficacy and dosing frequency of CCFA for the treatment of an infection caused by a specific pathogen is determined by the time DCA concentration in the affected tissue is above the MIC of ceftiolur for that specific pathogen. In cattle, a DCA concentration > 0.2 µg/mL maintained for at least 7 days has been efficacious for the treatment of respiratory disease.\textsuperscript{11,12,14} A DCA concentration ≥ 0.25 µg/mL is required to inhibit the growth of 90% of isolates of many common pathogens, including Streptococcus spp, A pleuropneumoniae, H parasuis, M haemolytica, Moraxella bovis, P multocida, Fusobacterium necrophorum, and Peptostreptococcus anaerobius of cattle and swine\textsuperscript{16-18} as well as for many respiratory pathogens of horses.\textsuperscript{24} In the present study, plasma DCA concentrations remained > 0.25 µg/mL for the entire 7-day study period, which suggested that a dose of 6.6 mg/kg, SC, in elephants should provide adequate protection against many pathogens for 7 to 10 days. The DCA concentration required to inhibit the growth of 90% of Escherichia coli isolates is 0.5 µg/mL, and that to inhibit the growth of 90% of Staphylococcus aureus and Salmonella spp isolates is 1 µg/mL.\textsuperscript{17} In the present study, mean plasma DCA concentrations were > 0.5 µg/mL for 6 days and > 1.0 µg/mL for 3 days.

For the elephants in the present study, CCFA absorption following SC administration was slow; the mean \(T_{\text{max}}\) was not detected until approximately 47 hours after administration and the mean ± SD \(C_{\text{max}}\) was only 1.36 ± 0.74 µg/mL. In contrast, for cattle that were administered the same dose (6.6 mg/kg) of CCFA as that administered to the elephants of the present study, the mean ± SD \(T_{\text{max}}\) for measured CFAEs was 12.0 ± 6.2 hours and the \(C_{\text{max}}\) was 6.9 ± 2.68 µg/mL.\textsuperscript{16} For swine that were administered a lower dose (5 mg/kg, SC) of CCFA than the elephants of the present study, the mean ± SD \(T_{\text{max}}\) for CFAEs was 22.0 ± 12.2 hours and the \(C_{\text{max}}\) was 4.17 ± 0.92 µg/mL.\textsuperscript{18} For the cattle and swine in those studies,\textsuperscript{16,18} the mean ± SD AUCs from time 0 to the lower LOQ were 376 ± 66.1 µg•h/mL and 373 ± 56.1 µg•h/mL, respectively, which were higher than the AUC\textsubscript{0-cmax} (228 ± 55.7 µg•h/mL) calculated for elephants in the present study. The mean extrapolated fraction of AUC\textsubscript{0-cmax} was 29.55% for elephants in the present study, slightly higher than what is considered optimal.\textsuperscript{25}

Ceftiolur (DCA) is eliminated primarily through the urinary system, although some is also eliminated by the biliary system.\textsuperscript{26} When a compound is absorbed slowly, the terminal half-life may not be a true representation of the elimination process because it is also affected by the ongoing absorption of the compound. Regardless, the mean terminal half-life of DCA calculated for elephants (83.36 ± 30.01 hours) in the present study was longer than the mean terminal half-lives of CFAEs calculated following administration of CCFA (6.6 mg/kg, SC) in the caudal portion of an ear of beef cattle (62.3 ± 13 hours)\textsuperscript{10} and administration of CCFA (5.0 mg/kg, IM) in swine (49.6 ± 11.8 hours).\textsuperscript{18}

For 2 of the elephants in the present study, plasma DCA concentrations had not substantially decreased at the conclusion of the study (168 hours after CCFA administration). Compared with results for the remainder of the study population, these elephants had the longest \(T_{\text{max}}\) and lowest \(C_{\text{max}}\), which indicated very slow absorption of CCFA in these 2 elephants. The results for those 2 elephants could also be explained by lower bioavailability of CCFA, but that could not be assessed without pharmacokinetic data following IV administration of ceftiolur. In the present study, the wide range for \(T_{\text{max}}\) (12 to 96 hours) was most likely caused by variability in the absorption of CCFA among elephants. A slow rate of CCFA absorption in elephants may be beneficial, especially when CCFA is used for the treatment of infections caused by bacteria with low MICs for ceftiolur because it may prolong the duration that plasma DCA concentration is above the MIC. Also, although not assessed in the present study, CCFA absorption in elephants may vary depending on the site of administration and whether the dose is administered in multiple locations.

Results of other studies\textsuperscript{21,22} indicate differences in the pharmacokinetics for some drugs when administered to Asian versus African elephants (Loxodonta africana). Although the study reported here was designed to evaluate the pharmacokinetic profile of CCFA in Asian elephants, our laboratory group has obtained comparable pharmacokinetic data following the administration of CCFA (6.6 mg/kg) SC in the loose skin of the caudoventral flank area of 2 African elephants. Evaluation of the data from the 2 African elephants revealed a similar mean ± SD \(C_{\text{max}}\) (0.85 ± 0.35 µg/mL), compared with the mean ± SD \(C_{\text{max}}\) (1.36 ± 0.74 µg/mL) for the Asian elephants of the present study. However the mean \(T_{\text{max}}\) (24 hours) for the African elephants was detected sooner than the mean \(T_{\text{max}}\) (47 hours) for the Asian elephants of the present study. The mean ± SD AUC\textsubscript{0-cmax} (94.5 ± 5.1 µg•h/mL) for DCA in the 2 African elephants was lower than the mean AUC\textsubscript{0-cmax} (145.77 ± 62.72 µg•h/mL) for DCA in the Asian elephants. For the 2 African elephants, the median terminal half-life of DCA (90.8 hours; harmonic mean ± pseudo-SD, 90 ± 28.1 hours) was similar to that (80.34; harmonic mean ± pseudo-SD, 83.36 ± 30.01 hours) for the Asian elephants of the present study. Although the pharmacokinetics for CCFA following SC administration may be similar between African and Asian elephants, these results should be evaluated with caution because of the limited number of African elephants sampled and the different site of CCFA administration (flank area vs ear area). Further research is necessary to elucidate the behavior of CCFA in both elephant species.

In the present study, the only adverse effect detected following CCFA administration was a reaction at the injection site in 3 elephants. Localized abscess formation following an SC or IM injection is common in elephants.\textsuperscript{9} The general recommendation for the maximum volume of drug that can be administered IM at 11
injection site is ≤ 15 to 25 mL in elephants.9,20 A similar recommendation for the maximum volume of a drug that can be administered SC at 1 injection site does not exist because SC administration of drugs to elephants has not been recommended due to variable drug absorption. In the present study, the entire CCFA volume (up to 140 mL) was administered at 1 site to elephants to decrease the number of variables that would need to be controlled. It is unknown whether a recommendation regarding the maximum volume of CCFA injected at a single site should be made because multiple injections will increase the risk for injection site contamination and subsequent abscess formation. Most of the elephants in the present study did not develop a reaction at the CCFA injection site, and for the 3 elephants that did develop a reaction (indurated plaque or abscess) at the CCFA injection site, the reaction resolved without complication. Also, the injection site reactions may have been inflammatory reactions to the large volume of the oil-based carrier of the CCFA that was injected rather than abscesses because cultures of aspirates from injection site plaques obtained from 2 of the study elephants resulted in no bacterial growth.

Generally, the administration of ceftiofur is associated with a wide margin of safety. As a class, cephalosporins have a low potential for the induction of nephrotoxicosis, and other adverse effects associated with cephalosporin administration are uncommon.21 In cattle, the inadvertent administration of CCFA into an artery within the pinna of an ear has been fatal.16,17 Although it is unlikely a similar scenario would occur in elephants, precautions should be taken to ensure that CCFA is not administered directly into a blood vessel of an elephant. In horses, ceftiofur sodium is well tolerated at up to 5 times the recommended dose for 3 times the recommended duration of treatment.28 Given the wide margin of safety and minimal adverse effects associated with cephalosporin administration, doses of ceftiofur > 6.6 mg/kg may be tolerated by elephants but further research is necessary before such recommendations can be made.

The fraction of DCA not bound to plasma proteins is unknown following SC administration of CCFA to elephants, but DCA binding to plasma proteins is readily reversible in other species.18 Therefore, our dosing recommendations for SC administration of CCFA to Asian elephants were made with the assumption that the plasma DCA concentration would remain greater than a given MIC for the entire interval between doses. On the basis of the results of the present study, a single 6.6 mg/kg dose of CCFA administered SC to Asian elephants will provide plasma DCA concentrations ≥ 0.25 µg/mL for 7 to 10 days and should be an effective treatment for infections caused by bacteria with MICs for ceftiofur ≤ 0.25 µg/mL. However, for the treatment of injections caused by bacteria with MICs for ceftiofur > 0.25 µg/mL, the same dose of CCFA would have to be administered more frequently. Cefiofur crystalline-free acid is not approved for use in elephants in the United States; therefore, CCFA was used in an extralabel manner in the present study. Results of bacterial susceptibility testing should be used whenever possible to guide decisions regarding the clinical use of CCFA (or any antimicrobial) because indiscriminate use of antimicrobials may result in the development of antimicrobial-resistant strains of bacteria.

a. Excede, 200 mg/mL, Pfizer Inc, New York, NY.
b. Tecan Freedom EVO, Raleigh, NC.
c. Isolute, 100 mg C18 SPE, Biotage, Uppsala, Sweden.
d. Waters Corp, Milford, Mass.
e. MDS Sciex 4000 Q Trap, Applied Biosystems, Foster City, Calif.
f. Turbolonspray, Applied Biosystems, Foster City, Calif.
g. Winnonlin, version 3.2.1, Pharsight Corp, Mountain View, Calif.

Reference